Research Paper

Simple Spectrophotometric Determination of Isoxsuprine Hydrochloride in Spiked Human Urine and in Tablets Using Folin-Ciocalteu's Reagent

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A very simple, rapid and cost effective spectrophotometric method is described for the determination of isoxsuprine hydrochloride (ISX). The method is based on the selective reduction of Folin-Ciocalteu's reagent (F-C reagent) by phenolic and amino group present in ISX in alkaline medium to form a blue coloured chromogen which absorbs maximally at 770 nm. Beer's law is obeyed in the concentration range 2.0-40.0 µg ml^{-1} with correlation coefficient, molar absorptivity and Sandell's sensitivity values of 0.9993, $6.61x10^3$ l mol^{-1} cm^{-1} and 0.0511µg cm^{-2}, respectively. Various experimental variables were optimized to obtain reliable results. The method was successfully applied to the determination of ISX in spiked human urine, and in pharmaceutical formulations. The results of analysis of the pharmaceuticals tallied well with the label claim and were statistically compared with those of the official method by applying the Student's t-test and F-test. The accuracy was further ascertained from spike-recovery method.

Key Words: Isoxsuprine Hydrochloride; Folin-Ciocalteu Reagent; Spiked Human Urine; Pharmaceuticals; Spectrophotometry

1. Introduction

Isoxsuprine hydrochloride (ISX) is chemically known as 4-Hydroxy-α-[1-[(1-methyl-2-phenoxyethyl) amino] ethyl] benzenemethanol hydrochloride (Fig. 1). ISX is a vasodilator and causes direct relaxation of vascular and uterine smooth muscle. Its use in the treatment of premature labour and peripheral vasodilator is well known [1]. The USP XXI [2] adopts UV spectrophotometric measurement of aqueous solution of ISX at about 269 and 300 nm, while the British pharmacopoeia [3] recommends a visual non-aqueous titration using $HClO_4$ and 1-naptholbenzein as indicator.

Different analytical techniques have been reported for the determination of ISX in both pharmaceuticals and biological matrices. These includes UV spectrophotometry [4, 5], ion selective electrode potentiometry [6], chemiluminescence [7], high performance liquid chromatography (HPLC) [8, 10], gas chromatography (GC) [11], GC-MS [12], LC-MS [13], affinity chromatography [14], polorography [16] and fluorimetry [17].

Due to the presence of vulnerable multi functional groups in ISX, viz., phenolic, secondary aliphatic amino and hydroxyl, the quantification of ISX has been accomplished by spectrophotometry based on a number of colour reactions [18-47]. But the reported methods suffer from one or the other disadvantage such as critical optimum conditions, heating and/or extraction step, multi step reaction, narrow linear dynamic range and/or low sensitivity and poor selectivity (Table 1).

Although the reaction between ISX and F-C reagent was exploited by Sane [31] *et al.*, for the determination of ISX in pharmaceuticals in NaOH medium, but the strong basicity of NaOH restricts quantitative extraction of ISX from the urine matrix due to interference. The use of Na_2CO_3, a milder base, by the present authors gave two advantages. Firstly, the ISX present in urine matrix was quantified with

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satisfactory percent recovery and secondly, the resulting chromophoric product shows absorption maximum at higher wavelength (770 nm). The proposed method exploits the analytical utility of blue colour chromogenic product, peaking at 770 nm, formed after the reduction of F-C reagent by phenolic and amino groups of ISX. The method is very simple, accurate and precise, has a wide linear dynamic range and selectively quantifies ISX in spiked human urine and in pharmaceuticals. Further, the proposed chemo selective method has many advantages over above the mentioned physico-methods [4-17] in terms of simplicity, cost-effectiveness and freedom from cumbersome instrumentations. The readers are requested to consult the comprehensive updates on all the methods reported for ISX since 1980s in recently published paper by Kalsang Tharpa *et al*. [48].

2. Materials and Methods

(a) Instrument

A Systronics Model 106 Digital Spectrophotometer provided with 1-cm matched quartz cells was used for absorbance measurements.

(b) Chemicals and Reagents

All chemicals were of analytical reagent grade and distilled water was used to prepare solutions.

A 0.05 M Folin-Ciocalteu's reagent solution was prepared by diluting 17.5 ml of the chemical (Merck, Mumbai, India, Sp. gr. 1.24, FW 8659) with 50 ml of water in a standard flask. The solution decomposition occurs at 2 per cent per month even kept under refrigeration [49], which does not affect within the present analytical frame work.

Carbonate buffer of pH 9.4 was prepared by dissolving 1.325g of Na_2CO_3 (Merck, Mumbai, India) and 1.050g of $NaHCO_3$ (Merck, Mumbai, India) in a 25 ml standard flask with water.

Ethyl acetate (Loba Chemie, Mumbai, India) was used as such.

10% Sodium carbonate solution was prepared in water.

(c) Standard Preparation

A stock standard solution of 500 µg ml^{-1} ISX was prepared by dissolving 50 mg ISX (Juggat Pharma, India, 99.86 ± 0.40% purity) in water, and made upto 100 ml with water in a calibrated flask. The stock solution is diluted to 100 µg ml^{-1} working concentration. An aqueous solution of ISX was reported to remain stable for 323 days [36].

(1) Procedure for Calibration Curve

Different concentrations (2.0-40.0 µg ml^{-1}) of standard solution of pure ISX were transferred into a series of 10 ml calibrated flasks by means of micro burette and the total volume was adjusted to 4.0 ml with water. To each flask, 2 ml of 0.05M solution of F-C reagent was added followed by 3 ml of 10% Na_2CO_3 and finally the volume was made up to the mark with water. The absorbance was measured after 10 minutes at 770 nm against a reagent blank.

(2) Procedure for Spiked Human Urine

Five ml of urine (ISX-free) was taken in a 250 ml separating funnel and spiked with 10 ml aqueous solution containing 2.5 mg of ISX. To the same solution, 5 ml of carbonate buffer of pH 9.48 was added followed by 20 ml of ethylacetate. The content was shaken for 15 minutes and the liquids were allowed to separate into two immiscible phase. The lower aqueous layer was discarded and the upper organic layer was collected in a beaker containing anhydrous sodium sulphate to remove residual water. Finally, water-free organic layer was transferred into a dried beaker and evaporated to dryness in a hot water bath. The dry residue was reconstituted with 2 ml of 1M HCl and transferred into a 25 ml calibrated flask. The volume was made up to the mark with water. An aliquot of resulting solution was analyzed using the procedure described under "*Procedure for Calibration Curve*" after suitable dilution.

(3) Procedure for Tablets

Twenty tablets each containing 20 or 40 mg of ISX were weighed and ground into a fine powder. An amount of powder equivalent to 50 mg of ISX was weighed into a 100 ml calibrated flask, 40 ml of water added and the mixture shaken for 20 min; then the volume was made up to the mark with same solvent, mixed well and filtered using Whatman No. 42 filter paper. The resulting 500 µg ml^{-1} ISX was further diluted to achieve working concentration of 100 µg ml^{-1} ISX and subjected to analysis using the procedure described under "*Procedure for Calibration Curve*".

3. Results and Discussion

Folin-Ciocalteu's reagent is a mixture of acids and involves the following chemical species:

$3H_2O.P_2O_5.13WO_3.5MoO_3.10H_2O$ and
$3H_2O.P_2O_5.14WO_3.4MoO_3.10H_2O$

This reagent is used extensively in the determination of large number of substances of pharmaceutical interest [50]. In the alkaline medium, ISX react with F-C reagent resulting in the formation of blue coloured chromogen which absorbs maximally at 770 nm (Fig. 2). The stoichiometry of the reaction was studied adopting the limiting logarithmic method [50]. Two straight lines were obtained upon using increasing concentrations of F-C reagent while keeping the concentration of ISX constant (Fig. 3a) and upon using increasing concentrations of ISX while keeping the concentration of the F-C reagent (Fig. 3b). The slopes of the two straight lines are 0.64 and 0.20. This means that the reaction proceeds in a molar ratio of 0.64:0.20, i.e. in a ratio of »3:1. Therefore, colour development is due to the transfer of three electrons from F-C reagent to ISX in basic medium to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower valence.

4. Optimization of Variables

The reaction proceeds only in basic medium. Different aqueous bases, such as sodium hydroxide, sodium carbonate or bicarbonate and sodium acetate were tested. A nearly instantaneous reaction (10 min) with a stable blue colour chromogen was achieved in sodium carbonate medium, which showed only 0.03 per cent decrease in the absorbance reading at 60th min, ten min after mixing the reactants (Fig. 4). The reaction requires 3 ml of 10% Na_2CO_3 (Fig. 5). Two ml of 0.05 M F-C reagent was found optimum and sensitivity of the reaction decreased at higher concentrations as shown in Fig. 6.

5. Method Validation

(a) Analytical Data

A linear correlation was found between absorbance and concentration of ISX in the range 2.0-40.0 µg ml^{-1} (Fig. 7). Linear regression analysis of the Beer's law data gave the equation A = (-0.0193 ± 0.0097) – (0.0179 ± 0.0002) C (r = 0.9993; n= 9) where A is the absorbance and C concentration in µg ml^{-1}. The limit of detection (LOD) and quantification (LOQ) calculated according to the current ICH guidelines [42] were 0.31 and 0.93 µg ml^{-1}, respectively. The calculated molar absorptivity was $6.61x10^3$ L mol^{-1} cm^{-1} and the Sandell sensitivity was 0.0511µg cm^{-2}.

(b) Precision and Accuracy

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [52]. Three different concentrations of ISX were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table 1). The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and taken concentrations for ISX (Bias %). The results obtained are compiled in Table 3 and show that the accuracy is good.

(c) Selectivity

A study of some potential interference in the present proposed methods was performed by selecting the excipients often used in pharmaceutical formulations

Table 1. Intra-day and Inter-day precision and accuracy evaluation

ISX µg ml^{-1} taken	Intra-day (n=7)			Inter-day (n=5)		
	ISX µg ml^{-1} found[a]	Precision[b]	Accuracy[c]	ISX µg ml^{-1} found[a]	Precision[b]	Accuracy[c]
15.0	15.12	0.96	0.80	15.14	1.10	0.93
25.0	25.69	1.52	2.76	26.0	1.30	4.00
35.0	34.64	0.46	1.02	34.50	0.75	1.43

a. Mean of n determinations, b. Relative standard deviation (%), c. Bias %: (found-taken/taken) x 100.

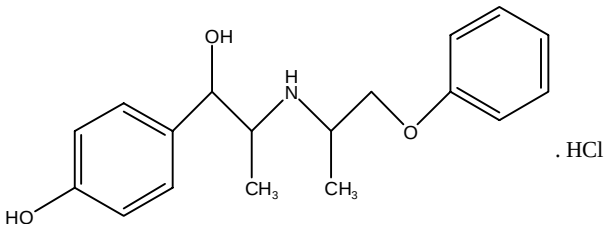


Fig. 1. Structure of isoxsuprine hydrochloride

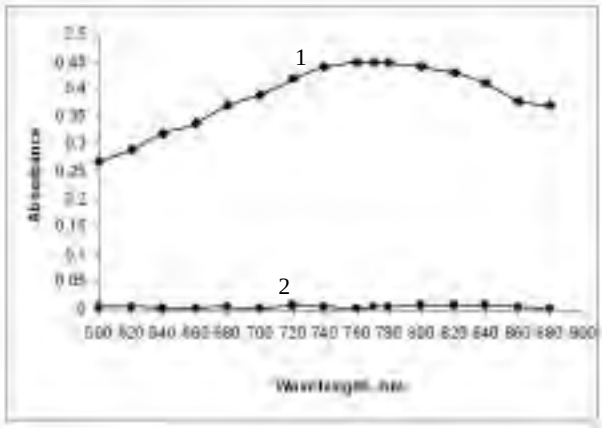
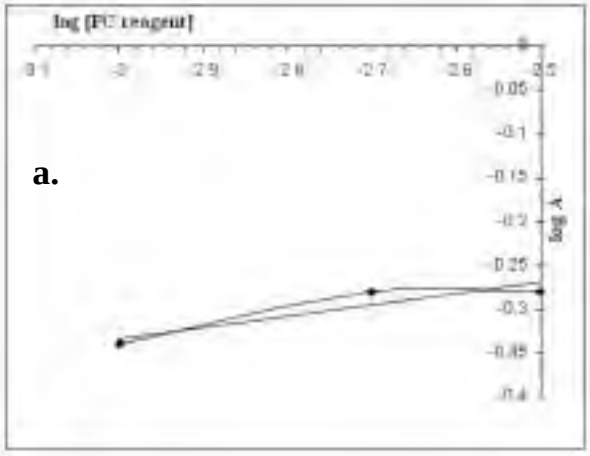


Fig. 2. Absorption spectra of- 1. Colour species (20 µg ml^{-1} ISX); 2. Reagent blank



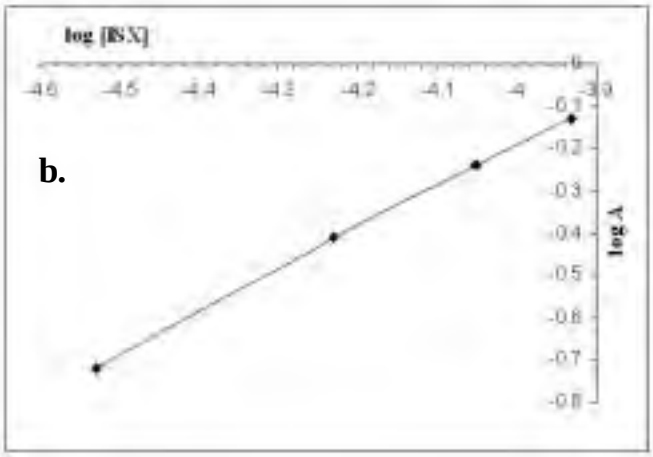


Fig. 3a. Limiting logarithmic plots for the molar reactivity of F-C reagent with ISX: log absorbance vs. log [FC reagent] at which [ISX] kept constant. b. log absorbance vs. log [ISX] at which [FC reagent] kept constant

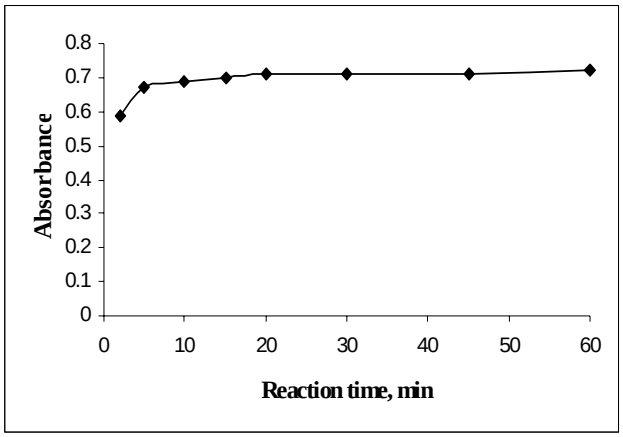


Fig. 4. Effect of time on colour formation (40 µg ml^{-1} ISX)

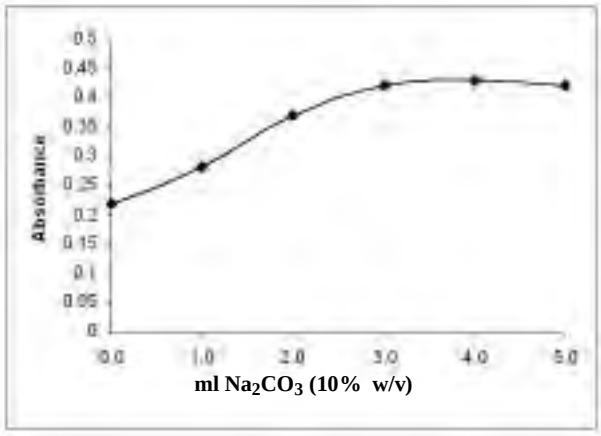


Fig. 5. Effect of 10% Na_2CO_3 on colour formation (20 µg ml^{-1} ISX)

or as possible co-active substance. Selectivity was evaluated by both placebo blank analysis and recovery studies. A placebo blank, the commonly employed tablet excipients, consisting of 20 mg sodium alginate, 30 mg magnesium stearate, 20 mg lactose, 20 mg acacia, 50 mg talc and 30 mg starch, but without ISX was prepared and analyzed as described under procedures. The resulting absorbance readings are same as reagent blank, inferring no interference from the placebo. It was further

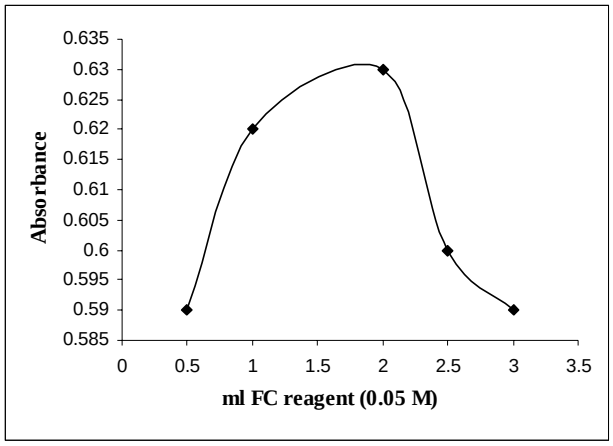


Fig. 6. Effect of F-C reagent (30 µg ml^{-1} ISX)

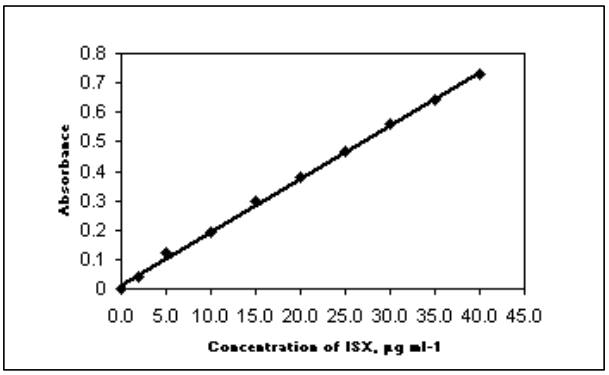


Fig. 7. Linear calibration plot

Table 2. Method robustness and ruggedness

ISX taken µg ml^{-1}	Robustness (% RSD)			Ruggedness (%RSD)	
	Reaction time[a]	X ml of 0.05 M F-C reagent	Y ml of 10 % Na_2CO_3	Inter instruments (n=3)	Inter analysts (n=4)
10.00	0.65	1.78	0.12	3.21	1.69
20.00	1.12	2.01	0.20	2.06	1.97
30.00	0.78	1.16	0.14	3.15	1.21
40.00	2.10	2.11	0.05	2.11	1.14

[a]Reaction time studied were 8.0, 10.0 and 12.0 min, X= 1.5, 2.0 and 2.2 ml and Y = 2.8, 3.0, 3.2 ml.

Table 3. Results of assay of tablets and statistical evaluation

Tablets claim	Label	Found[a] (Percent of analyzed label claim ±SD)	
		Reference method	Proposed method
Tidilan	20 mg/Tab	98.57 ± 0.76	99.96 ± 1.17 t=2.27 F= 2.37
Tidilan	40 mg/Tab	99.01 ± 1.08	96.57 ± 1.78 t=2.69 F=2.71

[a]Mean value of five determinations; Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39.

confirmed by carrying out recovery study from synthetic mixture prepared by adding 10 mg of ISX to 50 mg of the placebo blank. The active component was extracted into water as described under "procedure for tablets". The mean percent recovery of ISX was 101.24 ± 0.95 (n=5). This confirms the selectivity of method.

(d) Robustness and Ruggedness

For the evaluation of the method robustness, two important experimental variables such as reaction time and reagent concentration were slightly varied deliberately. The analysis was performed at the deliberately varied experimental conditions by taking four different concentrations of ISX and found to remain unaffected as shown by the RSD values in the range of 0.05 to 2.11 %. Method ruggedness was expressed as the RSD of the same procedure applied by four different analysts as well as using two different spectrophotometers. The results are shown in Table 4.

(e) Application to Analysis of Spiked Urine Sample and Pharmaceutical Formulations

The proposed methods were successfully applied to the determination of ISX in spiked urine sample with mean percent recovery of 97.55 ± 0.12 (n=5), and two representative tablets (Table 5). The results obtained were statistically compared with those of the official method [2] by applying the Students t-test for accuracy and F-test for precision. The official method consisted extraction of ISX from the matrices into aqueous solution and measured at 300 nm. As can be seen from the Table 5, the calculated t-value and F-value at 95% confidence level did not exceed

Table 4. Results of spike-recovery studies from pre-analyzed tablets

Formulation studied	ISX in tablet, µg ml^{-1}	Pure ISX added, µg ml^{-1}	Total found, µg ml^{-1}	Pure ISX recovered[a], Percent ± SD
Tidilan[b] 20 mg	10.00	5.000	15.60	103.3 ± 1.04
	10.00	10.00	20.10	101.0 ± 0.85
	10.00	15.00	25.77	105.1 ± 1.26
Tidilan[b] 40 mg	9.660	5.000	14.54	97.60 ± 0.92
	9.660	10.00	19.49	98.30 ± 1.12
	9.660	15.00	24.18	96.80 ± 0.83

[a]Mean value of three determinations; Marketed by: [b]Juggat Pharma, Bangalore 560074

the tabulated values of 2.77 and 6.39, respectively, for four degrees of freedom. The results indicate that there is no difference between the proposed methods and the official method with respect to accuracy and precision. Accuracy of the method was further confirmed by standard-addition procedure. Pre-analysed tablet powder (Tidilan 40 mg) was spiked with pure ISX at three different levels (50, 100 and 150% of the quantity present in the tablet powder) and the total was found by the proposed method. The results are presented in Table 4.

6. Conclusions

A very simple, rapid and cost effective yet versatile spectrophotometric method for the determination of ISX in spiked urine and pharmaceuticals is proposed. In contrast to the reported visible spectrophotometric methods, the proposed method is free from multi-step reactions, heating, pretreatment step, critical experimental conditions and use of expensive chemicals and/or organic solvents. Selectivity of the reaction is reflected in satisfactory recovery of ISX from urine matrix and in the presence of excipients in pharmaceuticals. Further, this reaction gives positive test on a Whatman filter paper which opens a possibility for an analyst to develop visible reflectance spectrophotometric procedure, which seems to be a recent trend in the realm of green analytical chemistry.

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References

1. Reynolds JEF (Ed.) (1996) 31st ed., Martindale, The Extra Pharmacopoeia, The pharmaceutical press, London
2. The United States Pharmacopoeia XXI (1984), pp 577, National Formulary 19, Rockville, USP Convention, USA
3. British Pharmacopoeia (1988) pp. 321, Vol. 1, Her Majesty's Stationary Office, London
4. Bryant R, Mantle DE, Timma DL, Yoder DS Single-tablet assays. Automated assay of single tablets of isoxsuprine hydrochloride. *J Pharm Sci* **57** (1968) 658-660
5. Cevdet D and Richard GB Multivariate calibration on designed mixtures of four pharmaceuticals. *Analyst* **123** (1998) 181-189
6. John CA, Constantinos AG and Michael AK Use of ion-selective electrodes in kinetic flow injection: Determination of phenolic and hydrazine drugs with 1-fluoro-2,4-dinitrobenzene using a fluoride-selective electrode. *Analyst* **116** (1991) 233-237
7. Aly FA and Salma AT Determination of phenolic sympathomimetric drugs in pharmaceuticals samples and biological fluids by flow injection chemiluminescence. *J AOAC Int* **83** (2000) 1299-1305
8. Ayman H and Benedikt L Determination of isoxsuprine in equine plasma by high-performance liquid chromatography with electrochemical detection. *J Chromatography B: Biomed Sci Appl* **563** (1991) 216-223
9. Belal F, Al-malaq HA, Al-majed AA and Gadkariem EA A stability-indicating HPLC method for the determination of isoxsuprine in dosage forms. Application to kinetic studies of isoxsuprine. *J Liq Chroma & Rel Tech* **23** (2000) 3175-3189
10. Volpe F, Zintel J and Spiegel D High-performance liquid chromatography of two peripheral vasodilators, nylidrin hydrochloride and isoxsuprine hydrochloride, in pharmaceutical dosage forms. *J Pharm Sci* **68** (1979) 1264-1267
11. Cova D, Colombo R and Cellini G Determination of isoxsuprine in human plasma by gas chromatography with electron capture detection. *Pharmacology* **27** (1983) 117-124
12. Bosken JM, Lehner AF, Hughes CG, Woods WE, Camargo FC, Harkins JD, Boyles J and Tobin T A GC-MS method for the determination of isoxsuprine in biological fluids of the horse utilizing electron impact ionization. *J Anal Toxicol* **28** (2004) 27-34
13. Kootstra PR, Kuijpers CJPF, Wubs KL, Van Doorn D, Sterk SS, Van Ginkel LA and Stephany RW The analysis of beta-agonists in bovine muscle using molecular imprinted polymers with ion trap LCMS screening. *Anal Chim Acta* **529** (2005) 75-81
14. Gianfranco B, Maurizio F, Ilenia C, Luigi S and Pasquale G α_1-acid glycoprotein affinity columns for the clean-up of adrenergic drugs. *Analyst* **123** (1998) 2693-2696
15. Belal F, AL-Malaq HA and AL-Majed AA Voltammetric determination of isoxsuprine and fenoterol in dosage forms and biological fluids through nitrosation. *J Pharm Biomed Anal* **23** (2000) 1005-1015

16. El-Enany N, Belal F and Rizk M Kinetic spectrophotometric determination of isoxsuprine in dosage forms through derivatisation with 4-chloro-7- nitrobenzo-2-oxa-1,3-diazole (nbd-cl). *Sci Pharm* **74** (2006) 99-119
17. Nawal AAA Fluorimetric determination of isoxsuprine hydrochloride in pharmaceuticals and biological fluids. *J Pharm Biomed Anal* **28** (2002) 331-335
18. Sane RT, Nayak VG and Malkar VB A simple spectrophotometric method for the determination of nylidrin hydrochloride, isoxsuprine hydrochloride and salbutamol sulphate in pharmaceutical preparations. *Talanta* **32** (1985) 31-33
19. Rajeswari CV, Naidu DV, Naidu NVS and Naidu PR A simple spectrophotometric method for determination of isoxsuprine hydrochloride in pharmaceuticals. *Talanta* **35** (1988) 237-238
20. Shingbal DM and Khanderparkar AS Application of p-amino phenol in the estimation of isoxsuprine hydrochloride and its dosage forms. *Indian Drugs* **25** (1988) 255-256
21. Constantinos GA and Michael AK Automated flow injection spectrophotometric determination of para- and meta- substituted phenols of pharmaceutical interest based on their oxidative condensation with 1-nitroso-2-napthol. *Analyst* **115** (1990) 309-313
22. Reddy MN, Sankar DG, Sekhar VV, Ravindra N and Rao GD Spectrophotometric determination of some adrenergic agents using iron (III) and 1, 10 phenanthroline. *Indian Drugs* **28** (1991) 331-333
23. Reddy MN and Sankar DG Spectrophotometric determination of isoxsuprine and nylidrin in pharmaceutical preparations. *Indian Drugs* **33** (1996) 420-422
24. Reddy MN, Sankar DG and Rao KVP Spectropho-tometric determination of isoxsuprine and nylidrin using 1, 10 phenanthroline. *Indian Drugs* **35** (1998) 163-164
25. Revanasiddappa HD and Manju BG Spectropho-tometric determination of ritodrine and isoxsuprine hydrochlorides using 4-aminoantipyrine. *J AOAC Int* **83** (2000) 1440-1445
26. El-Enany N, Belal F and Rizk M A simple kinetic spectrophotometric method for the determination of isoxsuprine in dosage forms. *II Farmaco* **57** (2002) 641-648
27. Negussie WB, Jacobus FVS, Raluca-Ioana S and Hassan YA Determination of isoxsuprine hydrochloride by sequential injection visible spectrophotometry. *II Farmaco* **60** (2005) 613-619
28. Rania SB, Abdel FW and Saied FB Spectrophotometric determination of etilefrine, ritodrine, isoxsuprine and salbutamol by nitration and subsequent meisenheimer complex formation. *Anal Lett* **28** (1995) 2503-2519
29. Shingbal DM and Velingker VS Colorimetric determination of isoxsuprine hydrochloride. *Indian J Pharm Sci* **42** (1980) 122-123
30. Sane RT, Nayak VG and Dhamankar AY Note on the estimation of isoxsuprine hydrochloride from pharmaceutical preparations. *Indian Drugs* **19** (1982) 284-286
31. Sane RT, Nayak VG, Joshi SK, Pandit UR and Sule UA Spectrophotometric method for determination of six drugs using Folin-Ciocalteu phenol reagent. *Indian Drugs* **20** (1983) 329-330
32. Shingbai DM and Agni RM Sensitive colorimetric method for estimation of isoxsuprine hydrochloride. *Indian Drugs* **20** (1983) 203-205
33. Shankar DG, Sastry CSP, Reddy MN and Singh NRP Spectrophotometric determination of some adrenergic drugs using 4-aminophenol. *Indian Drugs* **25** (1988) 478-479
34. Shingbal DM and Kudchadkar HS Colorimetric estimation of isoxsuprine hydrochloride in pharmaceuticals using copper chelation. *Indian Drugs* **24** (1987) 535-536
35. Chatterjee PK, Jain CL and Sethi PD Colorimetric determination of isoxsuprine hydrochloride through nitrosation and subsequent chelation. *Indian Drugs* **24** (1987) 210-213
36. Krishna G, Talwar SK, Sharma SC and Sharma RG Colorimetric determination of isoxsuprine hydrochloride in dosage forms. *East Pharm* **32** (1989) 189-190
37. Sankar DG, Sastry CSP, Reddy MN and Singh NRP Spectrophotometric determination of some adrenergic drugs with NN-dimethyl-p-phenylenediamine. *Indian Drugs* **24** (1987) 410-412
38. Guneri T and Sevgi F Spectrophotometric method for the determination of isoxsuprine hydrochloride. *Acta Pharm Turc* **32** (1990) 29-32
39. Sankar DG, Sastry CSP, Reddy MN and Aruna M Extraction spectrophotometric determination of some adrenergic drugs using fast green FCF or Orange II. *Indian Drugs* **26** (1989) 348-351
40. Sankar DG, Sastry CSP, Reddy MN and Prasad SNR Spectrophotometric determination of some adrenergic drugs using 2,6-dichloroquinone chlorimide [3,5-dichloro-p-benzoquinonechlorimine]. *Indian J Pharm Sci* **49** (1987) 69-71
41. Reddy MN, Sankar DG, Sreedhar K, Sankar PR and Rao GD New spectrophotometric method for the determination of some adrenergic agents. *Indian Drugs* **31** (1994) 109-111
42. Shingbal DM and Natekar GB Simple method for estimation of isoxsuprine hydrochloride. *East Pharm* **23** (1980) 109-110

43. Setty JT, Babu CS and Udupa N Spectrophotometric determination of isoxsuprine and nylidrin in pharmaceutical preparations. *Indian Drugs* **33** (1996) 124-126

44. Sankar DG, Sastry CSP, Reddy MN and Aruna M Spectrophotometric determination of some adrenergic agents using iron (III) and 2,4,6-tri-2-pyridyl-1,3,5-triazine. *Indian J Pharm Sci* **50** (1988) 178-180

45. Guneri T and Sevgi F Spectrophotometric determination of isoxsuprine hydrochloride**.** *Acta Pharm Turc* **30** (1988) 129-131

46. Anon Utility of 3-methylbenzothiazolin-2-one hydrazone in spectrophotometric determination of some adrenergic drugs. *Indian Drugs* **25** (1987) 130-132

47. Sane RT, Ambike VR, Joshi SK, Sawant SV, Doshi VJ, Nayak VG, Malkar VB, Pandit VR and Jukar S Application of molybdophosphoric acid in photometric determination of six drugs from pharmaceutical preparations. *Indian Drugs* **21** (1984) 254-256

48. Kalsang Tharpa, Basavaiah K, Revanasiddappa HD and Vinay KB Spectrophotometric determination of isoxsuprine hydrochloride using 3-methyl-2-benzothiazolinone hydrazone hydrochloride in spiked human urine and pharmaceuticals. *Talanta* **81** (2010) 1216-1223

49. Robert CP Stability of the Folin phenol reagent. *Acta Pharm Turc* **55** (2006) 523-524

50. Basavaiah K and Prammela HC Simple spectrophotometric determination of acyclovir in bulk drug and formulations. *II Farmaco* **57** (2002) 443-449

51. Rose J (1964) *Advanced Physico-chemical Experiments* Pitman and Sons, London, pp. 67

52. International Conference on Hormonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonised Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R 1), Complementary Guideline on Methodology 06 Nov 1996 (incorporated in November 2005, London)